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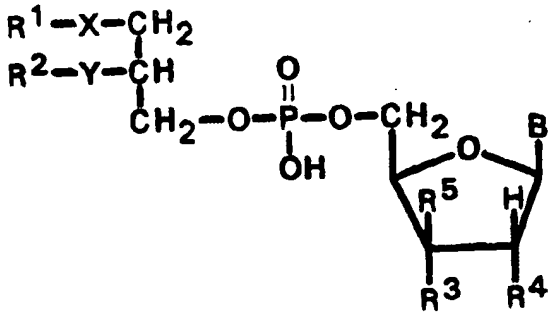
(57) Abstract

The present invention is directed to new nucleoside monophosphate derivatives of lipid ester residues of general formula (I), wherein R^1 represents an optionally substituted alkyl chain having 1-20 carbon atoms; R^2 represents hydrogen, an optionally substituted alkyl chain having 1-20 carbon atoms; R^3, R^4 and R^5 represent hydrogen, hydroxy, azido, amino, cyano, or halogen; X represents a valence dash, oxygen, sulfur, a sulfinyl or sulfonyl group; Y represents a valence dash, an oxygen or sulfur atom; B represents a purine and/or pyrimidine base; with the proviso that at least one of the residues R^3 or R^5 is hydrogen; to their tautomers and their physiologically acceptable salts of inorganic and organic acids and/or bases, as well as to processes for their preparation, and to drugs containing said compounds.



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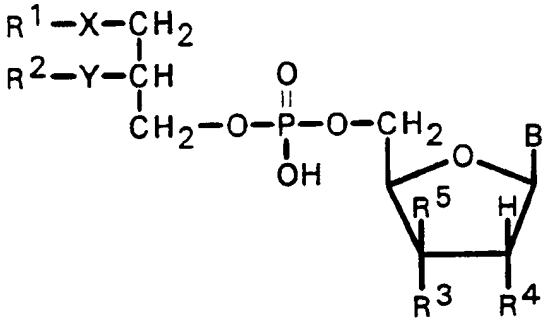
New Lipid Esters of Nucleoside Monophosphates and Their Use as Immunosuppressive Drugs

The present invention is directed to new nucleoside monophosphate derivatives of lipid ester residues of general formula (I)



(I),

wherein

R^1 may be a straight-chain or branched, saturated or unsaturated alkyl chain having 1-20 carbon atoms, optionally mono- or polysubstituted by halogen, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkylsulfinyl, or C_1-C_6 alkylsulfonyl groups;

R^2 may be hydrogen, a straight-chain or branched, saturated or unsaturated alkyl chain having 1-20 carbon atoms, optionally mono- or polysubstituted by halogen, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, C_1-C_6 alkoxycarbonyl, or C_1-C_6 alkylsulfonyl groups;

R^3 represents hydrogen, hydroxy, azido, amino, cyano, or halogen;

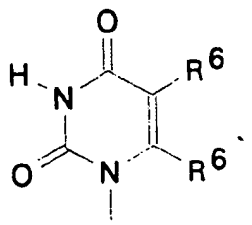
R^4 represents hydroxy, azido, amino, cyano, or halogen;

R^5 represents hydrogen, hydroxy, azido, amino, cyano, or halogen;

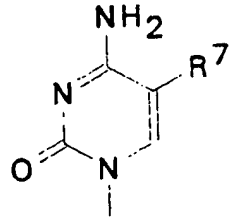
X represents a valence dash, oxygen, sulfur, a sulfinyl or sulfonyl group;

Y is a valence dash, an oxygen or sulfur atom;

B represents a purine and/or pyrimidine base of formula III(a-d)



(IIIa)



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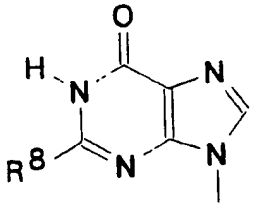

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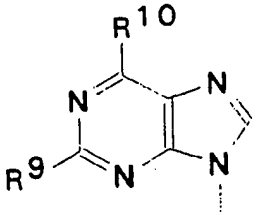


(IIId)

wherein

R^6 may be hydrogen; an alkyl chain having 1-6 carbon atoms, which may be substituted by halogen; an alkenyl and/or alkinyl residue having 2-6 carbon atoms, optionally substituted by halogen; or halogen;

$R^{6'}$ may be a hydrogen atom or a benzyl or phenylthio residue;

R^7 may be hydrogen; an alkyl chain having 1-6 carbon atoms, which may be substituted by halogen; or halogen;

R^8 may be hydrogen, an alkyl chain having 1-6 carbon atoms, halogen, or a hydroxy or an amino group;

R^9 may be hydrogen, an amino group or a halogen atom; and

R^{10} may be hydrogen, halogen, mercapto, hydroxy, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, or an amino group which may be mono- or disubstituted by C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy-C_2-C_6 alkyl, and/or C_3-C_6 cycloalkyl, aryl, hetaryl, aralkyl, or hetarylalkyl groups, optionally substituted at the aryl or hetaryl residue by one or more mercapto, hydroxy, C_1-C_6 alkoxy, or C_1-C_6 alkyl groups or halogen; or C_2-C_6 alkenyl optionally substituted by mono- or dialkyl or alkoxy groups;

with the proviso that at least one of the residues R^3 or R^5 is hydrogen;

to their tautomers and their physiologically acceptable salts of inorganic and organic acids and/or bases, as well as to processes for their preparation, and to drugs containing said compounds.

As these compounds of general formula I contain asymmetric carbon atoms, the invention is likewise directed to all the optically active forms and racemic mixtures of said compounds.

J. Biol. Chem. 265, 6112 (1990), and EP 0,350,287 describe preparation and use of liponucleotides as antiviral drugs. Therein, however, only dimyristoylphosphati-

dyl and dipalmitoylphosphatidyl residues coupled to familiar nucleosides such as AZT (azidothymidine) and ddC (2',3'-dideoxycytidine) have been examined and synthesized, including their fatty acid ester structure.

J. Med. Chem. 33, 1380 (1990), describes nucleoside conjugates of thioether lipides with cytidine diphosphate, which have antitumor activity and might find use in oncology. Chem. Pharm. Bull. 36, 209 (1988), describes 5'-(3-sn-phosphatidyl)nucleosides having antileukemic activity, as well as their enzymatic synthesis from the corresponding nucleosides and phosphocholines in the presence of phospholipase D with transferase activity. Similarly, J. Med. Chem. 34, 1408 (1991), describes nucleoside conjugates having anti-HIV 1 activity, which are substituted by methoxy or ethoxy in sn-2 position of the lipid portion. The patent application WO 92/03462 describes thioether lipid conjugates having antiviral activity, particularly for treating HIV infections.

The compounds of the present invention have valuable pharmacological properties. In particular, they are suitable in therapy and prophylaxis of malignant tumors such as malignancies, neoplasms, carcinomas, sarcomas, or leukemias in tumor therapy. In addition, the compounds exhibit immunosuppressive activity and therefore, they may be employed in the therapy of organ-specific or generalized auto-immune diseases such as rheumatoid arthritis, systemic lupus erythematosus, chronic graft vs. host disease, multiple sclerosis, etc., or in preventing allogenic or semiallogenic graft rejection, e.g., kidneys, liver, lungs,

heart, etc.. Furthermore, the compounds have antiviral, anti-retroviral or anti-oncogenic activity and thus, are also suitable in prophylaxis and therapy of viral and oncogenic-induced/caused diseases (such as AIDS etc.). Compared to compounds hitherto employed in treatment of malign tumors, the compounds according to the invention have enhanced efficacy or lower toxicity and thus, have a wider therapeutic range. For this reason, they are advantageous in that the administration of drugs containing these compounds may be conducted continuously over a prolonged period of time, and withdrawal of the preparation or intermittent administration, which frequently has been routine with cytostatic agents hitherto employed in tumor therapy or, due to their undesirable side-effects, has been necessary, can be avoided.

The compounds according to the invention do not suffer from these drawbacks. Their action is immunosuppressive or antitumoral, without being unspecifically cytotoxic in pharmacologically relevant doses.

Similarly, the compounds of the present invention and their pharmaceutical formulations may be employed in combination with other drugs for the treatment and prophylaxis of the diseases mentioned above. Examples of these further drugs involve agents such as, e.g., mitosis inhibitors such as colchicine, mitopodozid, vinblastine, alkylating cytostatic agents such as cyclophosphamide, melphalan, myleran or cisplatin, antimetabolites such as folic acid antagonists (methotrexate) and antagonists of purine and pyrimidine bases (mercaptopurine, 5-fluorouridine, cytarabin), cytostatically active antibiotics such as anthracyclines (e.g., doxorubicin, daunorubicin), hormones such as fosfestrol, tamoxifen, other cytostatically/cytotoxically active chemotherapeutic agents and other immunosuppressive drugs (such as cyclosporines, FK 506, rapamycines, desoxyspergualin, etc.).

Above all, possible salts of the compounds of general formula I are the alkali, alkaline earth and ammonium salts of the phosphate group. Preferred as the alkali salts are lithium, sodium and potassium salts. Possible as the alkaline earth salts are magnesium and calcium, in particular. According to the invention, ammonium salts are understood to be those containing the ammonium ion which may be substituted up to four times by alkyl residues having 1-4 carbon atoms, and/or aralkyl residues, preferably benzyl residues. Here, the substituents may be the same or different.

The compounds of general formula I may contain basic groups, particularly amino groups, which may be converted to acid addition salts by suitable inorganic or organic acids. To this end, possible as the acids are, in particular: hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, fumaric acid, succinic acid, tartaric acid, citric acid, lactic acid, maleic acid, or methanesulfonic acid.

In the general formula I, R^1 preferably represents a straight-chain C_8-C_{15} alkyl group which may be further substituted by a C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto group. More specifically, R^1 represents a nonyl, decyl, undecyl, dodecyl, tridecyl, or tetradecyl group. Preferably, methoxy, ethoxy, butoxy, and hexyloxy groups are possible as the C_1-C_6 alkoxy substituents of R^1. In case R^1 is substituted by a C_1-C_6 alkylmercapto residue, this is understood to be the methylmercapto, ethylmercapto, propylmercapto, butylmercapto, and hexylmercapto residue, in particular.

Preferably, R^2 represents a straight-chain C_8-C_{15} alkyl group which may be further substituted by a C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto group. More speci-

fically, R^2 represents an octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, or tetradecyl group. Preferably, methoxy, ethoxy, propoxy, butoxy, and hexyloxy groups are preferable as the C_1-C_6 alkoxy substituents of R^2. In case R^1 is substituted by a C_1-C_6 alkylmercapto residue, this is understood to be the methylmercapto, ethylmercapto, butylmercapto, and hexylmercapto residue, in particular.

Preferably, X is sulfur, sulfinyl or sulfonyl, and Y is oxygen.

Similarly, compounds are preferred, wherein X and Y represent a valence dash, R^2 is hydrogen, and R^1 represents a C_1-C_{20} alkyl chain optionally substituted by C_1-C_6 alkoxy or C_1-C_6 alkylmercapto.

Preferably, R^5 represents hydrogen, azido, cyano or halogen, such as fluorine, chlorine or bromine.

Preferably, each R^3 and R^4 represent a hydroxy or a cyano or azido group, or a halogen atom, such as fluorine, chlorine, bromine or iodine, wherein the residues may be the same or different.

Particularly preferred are compounds, wherein R^5 represents a hydrogen atom and R^3 and R^4 are hydroxy, cyano, azido or fluorine.

In the bases of general formula (III) the residues R^6 and R^7 preferably represent a hydrogen atom, a methyl, trifluoromethyl, ethyl, propyl, or butyl residue, or a halogen atom, such as fluorine, chlorine, bromine or iodine, as well as an alkenyl and/or alkinyl group which may be substituted by halogen.

Particularly preferred for R^6 and R^7 is a hydrogen atom, the methyl, trifluoromethyl or ethyl residues, and a fluorine, chlorine or bromine atom, and/or the vinyl, propenyl, ethinyl or propinyl residues optionally substituted by halogen.

Preferably, the residue R^8 is a hydrogen atom, a methyl, ethyl, propyl, or butyl residue, an amino group or a halogen atom such as fluorine, chlorine bromine or iodine, preferably chlorine or bromine.

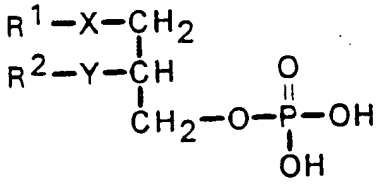
Preferably, R^{10} represents a hydrogen, fluorine, chlorine or bromine atom, a C_1-C_6 alkoxy group, more specifically a methoxy, ethoxy, propoxy, butoxy, or hexyloxy group, a mercapto residue, a C_1-C_6 alkylmercapto group, more specifically a methylmercapto, ethylmercapto, butylmercapto, or hexylmercapto group, or an amino group which may be mono- or disubstituted by a C_1-C_6 alkyl group, such as the methyl, ethyl, butyl or hexyl groups, by a hydroxy-C_2-C_6 alkyl group, such as the hydroxyethyl, hydroxypropyl, hydroxybutyl, or hydroxyhexyl groups, by a C_3-C_6 cycloalkyl residue, such as the cyclopropyl, cyclopentyl or cyclohexyl residues, by aryl, preferably phenyl, by an aralkyl residue, such as, in particular, benzyl optionally substituted by one or more hydroxy or methoxy groups, by C_1-C_6 alkyl groups, such as the methyl, ethyl, propyl, butyl, or hexyl groups, or by halogen atoms, such as fluorine, chlorine or bromine. Similarly, the amino group may be substituted by a hetarylalkyl or hetaryl residue such as, in particular, the thienyl, furyl or pyridyl residues, for example. Preferably, the hetaryl residue is understood to be the thienylmethyl, furylmethyl or pyridylmethyl residue.

Preferably, the following nucleosides are suitable as the coupling components to prepare the lipid-nucleotide conjugates of formula (I):

6-Mercaptopurine-9-β-D-ribofuranoside
5-Fluorouridine
Inosine
5-Methyluridine
2',3'-Didesoxy-2',3'-difluorothymidine
5-Chlorouridine
5-Trifluoromethyluridine
5-Ethinyluridine
5-Ethinylcytidine
5-Prop-1-enyluridine
5-Prop-2-enyluridine
Adenosine
Guanosine
2,6-Diaminopurine-9-β-D-ribofuranoside
2-Amino-6-mercaptopurine-9-ß-D-ribofuranoside
2-Amino-6-mercaptomethylpurine-9-ß-D-ribofuranoside
2-Amino-6-chloropurine-9-ß-D-ribofuranoside
2'-Desoxy-2'-aminoadenosine
2'-Desoxy-2'-azidoadenosine
2'-Desoxy-2'-azidocytidine
2'-Desoxy-5-fluorouridine
2-Chloroadenosine
2-Bromoadenosine
3'-Desoxy-3'-fluoroadenosine
6-Methylmercaptopurine-9-ß-D-ribofuranoside
2-Fluoroadenosine
2-Fluoro-2'-desoxyadenosine

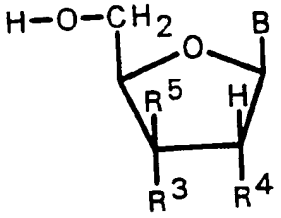
The compounds of general formula (I) may be prepared by

1. reacting a compound of general formula V



(V),

wherein R^1, R^2, X and Y have the meanings as indicated, with a compound of general formula VI

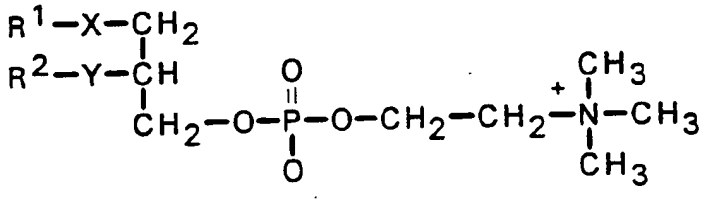


(VI),

wherin R^3, R^4, R^5 and B have the above-mentioned meanings, or represent a hydroxy group protected by an oxygen protecting group familiar to the artisan,

in the presence of an activating acid chloride, such as 2,4,6-triisopropylbenzenesulfonic acid chloride, and a tertiary nitrogen base, e.g., pyridine or lutidine, in an inert solvent, such as toluene, or immediately in anhydrous pyridine, and optionally, subsequent to hydrolysis, removing the oxygen protecting groups according to procedures conventional in nucleoside chemistry, or

2. reacting a compound of general formula VII



(VII),

wherein R^1, R^2, X and Y have the above-mentioned meanings, with a compound of general formula VI, wherein R^3, R^4, R^5 and B have the above-mentioned meanings, in the presence of phospholipase D from *Streptomyces* in an inert solvent such as chloroform, in the presence of a suitable buffer, and optionally, subsequent to reaction, removing the oxygen protecting groups according to procedures conventional in nucleoside chemistry.

The preparation of the compounds of general formula V and VII is performed in analogy to Lipids 22, 947 (1987), and J. Med. Chem. 34, 1377 (1991).

The preparation of the compounds of general formula VI is described, e.g., in EP-A-0,286,028 and WO 90/08147. Some of the included nucleosides are commercially available compounds.

Compounds similar to formula I are described in EP-A-0,350,287. Therein, the corresponding 1,2-diesters of glycerol are described.

The drugs containing compounds of formula I for the treatment of viral infections may be applied in liquid or solid forms on the intestinal or parenteral route. Here, the common application forms are possible, such as tablets, capsules, coated tablets, syrups, solutions, or suspensions. Preferably, water is used as the injection medium, containing additives such as stabilizers, solubilizers and buffers as are common with injection solutions. Such additives are, e.g., tartrate and citrate buffers, ethanol, complexing agents such as ethylenediaminetetraacetic acid and its non-toxic salts, high-molecular polymers such as liquid polyethylene oxide for viscosity control. Liquid vehicles for injection solutions need to be sterile and are filled in ampoules, preferably. Solid car-

riers are, for example, starch, lactose, mannitol, methylcellulose, talc, highly dispersed silicic acids, higher-molecular fatty acids such as stearic acid, gelatine, agar-agar, calcium phosphate, magnesium stearate, animal and plant fats, solid high-molecular polymers such as polyethylene glycol, etc.. If desired, formulations suitable for oral application may include flavorings or sweeteners.

The dosage may depend on various factors such as mode of application, species, age, or individual condition. Conventionally, the compounds according to the invention are applied in amounts of 0.1-100 mg, preferably 0.2-80 mg per day and per kg of body weight. It is preferred to divide the daily dose into 2-5 applications, with tablets having an active ingredient content of 0.5-500 mg being administered with each application. Similarly, the tablets may have sustained release, reducing the number of applications to 1-3 per day. The active ingredient content of sustained-release tablets may be 2-1000 mg. The active ingredient may also be administered by continuous infusions, where amounts of 5-1000 mg per day are normally sufficient.

In addition to the compounds mentioned in the examples, the following compounds of formula I are possible in the meaning of the present invention:

1. (5-Chlorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
2. (5-Trifluoromethyluridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
3. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
4. (5-Fluorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester

5. (5-Prop-1-enyluridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
6. (5-Ethinylcytidine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
7. (2-Amino-6-mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
8. (2,6-Diaminopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
9. (5-Prop-2-enyluridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
10. (5-Fluorouridine)-5'-phosphoric acid (3-dodecylsulfonyl-2-decyloxy)propyl ester
11. (5-Chlorouridine)-5'-phosphoric acid (3-dodecylsulfonyl-2-decyloxy)propyl ester
12. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylsulfonyl-2-decyloxy)propyl ester
13. (5-Fluorouridine)-5'-phosphoric acid (3-dodecyloxy-2-decyloxy)propyl ester
14. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecyloxy-2-decyloxy)propyl ester
15. (5-Fluorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decylmercapto)propyl ester
16. (5-Fluorouridine)-5'-phosphoric acid (3-undecylmercapto-2-undecyloxy)propyl ester
17. (5-Trifluoromethyluridine)-5'-phosphoric acid (3-undecylmercapto-2-undecyloxy)propyl ester
18. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-undecylmercapto-2-undecyloxy)propyl ester
19. (5-Trifluoromethyluridine)-5'-phosphoric acid (3-decylmercapto-2-dodecyloxy)propyl ester
20. (5-Fluorouridine)-5'-phosphoric acid (3-undecylmercapto-2-dodecyloxy)propyl ester
21. (5-Trifluoromethyluridine)-5'-phosphoric acid (3-undecylmercapto-2-decyloxy)propyl ester
22. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-tetradecylmercapto-2-decyloxy)propyl ester

23. (5-Fluorouridine)-5'-phosphoric acid (3-tridecylmercapto-2-decyloxy)propyl ester
24. (2-Fluoroadenosine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
25. (2-Desoxy-2-fluoroadenosine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
26. (6-Mercaptopurine)-9-ß-D-ribofuranoside)-5'-phosphoric acid dodecyl ester
27. (5-Fluorouridine)-5'-phosphoric acid hexadecyl ester
28. (5-Trifluoromethyluridine)-5'-phosphoric acid eicosyl ester
29. (5-Fluorouridine)-5'-phosphoric acid dodecyl ester
30. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid dodecyl ester

Example 1

(5-Fluorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester

3.6 g (6.1 mmoles) of phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester was treated twice with 30 ml of anhydrous pyridine and concentrated by evaporation. The residue was dissolved in 30 ml of anhydrous pyridine, treated with 2.76 g (9.1 mmoles) of 2,4,6-triisopropylbenzenesulfonic acid chloride under nitrogen and stirred at room temperature for 30 minutes. Then, 1.60 g (6.1 mmoles) of 5-fluorouridine (Fluka) was added, and the charge was allowed to stand under N_2 for 24 hours.

Hydrolysis was performed using 15 ml of water, the mixture was stirred for another 2 hours at room temperature, freed from solvent under vacuum, and stripped twice using a small amount of toluene. The residue was purified by column chromatography on LiChroprep® RP-18 with a linear

gradient of methanol/water 7/1 to methanol as the eluant. The yield is 3.1 g (69% of theoretical amount); oil. R_f = 0.24 (CH_2Cl_2/MeOH 8/2); R_f = 0.55 (CH_2Cl_2/MeOH/H_2O 6.5/2.5/0.4) on Merck 5715 TLC plates, silica gel 60 F.

The phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester was prepared as described in WO 92/03462.

Example 2

(6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester

6.2 g (12.5 mmoles) of phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester was treated with 5.7 g (18.75 mmoles) of 2,4,6-triisopropylbenzenesulfonic acid chloride as described in Example 1 and subsequently with 3.55 g (11.25 mmoles) of 6-mercaptopurine-9-ß-D-ribofuranoside and after 24 hours, this was hydrolyzed with water.

Then, 2.85 g of calcium acetate in 15 ml of water was slowly dropped therein, precipitating the crude calcium salt of the conjugate. After prolonged stirring the precipitate with acetone (1/10), 6 g of an amorphous crude product was obtained, having 72 area -% according to HPLC.

The calcium salt was suspended in 350 ml of methanol, treated with 150 g of Amberlite IR 120 in the Na^+ form and stirred for 2 days.

Thereafter, the ion exchanger was removed, the filtrate was evaporated, and the residue was purified by column chromatography on LiChroprep® RP-18 with a linear gradient of methanol/water 5/1 to 9/1. The fractions containing

product were evaporated in a vacuum, and the residue was stirred with acetone and dried. Yield: 3.52 g (41% of theoretical amount).

DC: R_f = 0.45 (isopropanol/butyl acetate/conc. ammonia/water 50/30/5/15).

Example 3

(6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester sodium salt

Analogous to Example 2, 41.4 g of phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester in 400 ml of anhydrous pyridine was reacted with 42.9 g of 2,4,6-triisopropylbenzenesulfonic acid chloride and subsequently with 23.7 g of 6-mercaptopurine-9-ß-D-ribofuranoside. The crude calcium salt which was filtered by suction after hydrolysis and precipitation with 25 g of calcium acetate in 160 ml of water, was distributed between 500 ml of MTB and 250 ml of 2N HCl and stirred until completely dissolved in the organic phase. The organic phase was separated, washed with saturated sodium chloride solution and concentrated in a rotary evaporator. The residue was applied onto 80 g of LiChroprep RP-18 (treat MTB solution of crude product with RP-18, evaporate and dry), and separated portion by portion in a pre-column on RP-18. Each time, a mixture of 3.7 l of methanol, 400 ml of water, 3 ml of glacial acetic acid, and 2 g of sodium acetate served as the eluant. The fractions containing product were combined, the desired compound was precipitated by adding 20 g of calcium acetate in 100 ml of water and filtered by suction. Yield: 32 g (43% of theoretical amount).

The calcium salt was suspended in 250 ml of MTB, extracted with 80 ml of 2N HCl by shaking, and the organic phase was washed twice with saturated sodium chloride solution. Following removal of the solvent, the residue was dissolved in 200 ml of toluene and adjusted to pH 7 against a Friscolyt electrode with 30% sodium methylate solution. The sodium salt was precipitated by stirring into 200 ml of acetone, filtered by suction and dried in a vacuum drying oven. Yield: 29 g (37% of theoretical amount).

R_f value: 0.18 (Silica gel; eluant: isopropanol/butyl acetate/water/conc. ammonia 50/30/15/5).

Example 4

(6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester sodium salt

Analogous to Example 3, the crude conjugate was prepared from 40 g of 6-mercaptopurine-9-ß-D-ribofuranoside. The crude product was purified by column chromatography using 8 g each time, on a column with DIOL phase (diameter 4 cm; length 25 cm) (detection at 254 nm; eluant: methanol/MTB 10/4). The applied sample had clearly dissolved in the eluant. The product-containing fractions of the different separations were combined, evaporated and precipitated as the sodium salt from toluene and acetone as in Example 3. Yield: 64.5 g (51% of theoretical amount).

R_f value: 0.85 (DIOL phase; eluant: methanol).

Example 5

(6-Methylmercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester sodium salt

Analogous to Example 1, 14.9 g of 6-methylmercaptopurine-9-ß-D-ribofuranoside (50 mmoles) were reacted with the mixed anhydride prepared from 27.3 g of phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester and 25 g of 2,4,6-triisopropylbenzenesulfonic acid chloride in 250 ml of anhydrous pyridine, hydrolyzed and concentrated by evaporation. Analogous to Example 3, the crude product (HPLC: 67 area %) was purified by chromatography on RP-18, precipitated as the calcium salt, and converted to the sodium salt. Yield: 15.2 g (38% of theoretical amount).

R_f value: 0.22 (Silica gel; eluant: isopropanol/butyl acetate/water/conc. ammonia 50/30/15/5).

Example 6

(5-Fluorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester sodium salt

Analogous to Example 1, 50 g of 5-fluorouridine was converted to the crude conjugate, precipitated as the calcium salt as described in Example 3 and subsequent to conversion to the free acid, was purified as the crude product by chromatography, analogous to Example 4, on a DIOL phase using methanol/MTB 10/4 as the eluant. The sodium salt prepared as in Example 3 was isolated in a yield of 69%.

R_f value: 0.35 (DIOL plates; eluant: methanol/MTB 10/4).

Example 7

IC_{50} values (µg/ml) for Azathioprine, 6-Mercaptopurine (6- MP), 6-Mercaptopurinribosid, BM 92.0729 and Doxorubicin in CFU-E and CFU-GM assays

This table shows the IC_{50} values (µg/ml) for Azathioprine, 6-Mercaptopurine (6-MP) and 6-Mercaptopurinribosid in comparison to the 6-Mercaptopurinribosid ether lipid conjugate BM 92.0729 for in vitro cytotoxicity on murine bone marrow stern cells, including colony-forming units/erythrocytes (CFU-E) and colony-forming units/granulocytes-macrophages (CFU-GM). The cytostatic/cytotoxic compound Doxorubicin was also included as reference substance. All compounds were tested in 3-6 different experiments concentration dependently with,at least, doublicate or triplicate inclubations per concentration tested.

As can be seen from the results, BM 92.0700 is much better tolerated by the bone marrow stern cells compared to all other compounds tested, in particular, in comparison to 6-Mercaptopurinribosid.

IC_{50} values (µg/ml) for Azathioprine, 6-Mercaptopurine (6-MP), 6-Mercaptopurinribosid, BM 92.0729 and Doxorubicin in CFU-E and CFU-GM assays.[a]

Compound	CFU-E			CFU-GM		
Azathioprine	0.0004	± 0.0001	(4)	0.0043	± 0.0019	(3)
6-MP	0.0003	± 0.0001	(4)	0.0023	± 0.00009	(3)
6-MP-Ribosid	0.0003	± 0.0001	(4)	0.0023	± 0.00013	(3)
BM 92.0729	0.056	± 0.013	(5)	0.247	± 0.044	(6)
Doxorubicin	0.0017	± 0.0005	(4)	0.050	± 0.004	(4)

[a] Mean ± SEM; n, number of different experiments.

Example 8

Bone marrow toxicity of BM 92.0729, Azathioprine, 6-Mercaptopurine and 6-Mercaptopurinribosid in female Balb/c mice: Day + 4 (Exp. 930740)

Exp. 930740 shows the bone marrow toxicity of BM 92.0792, Azathioprine, , 6-Mercaptopurine and 6-Mercaptopurinribosid in vivo in female Balb/c mice which were treated once daily p.o. for four consecutive days (day 0-day +3). The animals were killed on day +4 and bone marrow cellularity (cells/fernur) was determined. The results indicate no bone marrow toxicity for the 6-Mercaptopurinribosid ether lipid conjugate BM 92.0729 up to the highest dose tested, i.e. 100 $mg \cdot kg^{-1} \cdot day^{-1}$ which correspondenz on a molar basis with 30 $mg \cdot kg^{-1} \cdot day^{-1}$ of 6-Mercaptopurinribosid . This latter compond shows, in contrast to the ether lipid conjugate BM 92.0792, clearly a dose-dependent reduciton in one marrow cellularity. The same finding was obtained for the other substances, including Azathioprine and 6-Mercaptopurine .

Bone marrow toxicity of BM 92.0729, Azathioprine, 6-Mercaptopurine and 6-Mercaptopurinribosid in female Balb/c mice: Day + 4 (Exp. 930740)

Compound	Dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	Cells/femur (10^6)	
Control (0,5% Tylose)	-	15.9 ± 1.4 (8)	[a]
Azathioprine	10	11.6 ± 0.4 (9)	*
Azathioprine	30	9.6 ± 0.9 (9)	**
6-Mercaptopurine	10	13.0 ± 1.5 (8)	
6-Mercaptopurine	30	6.5 ± 0.7 (9)	**
6-Mercaptopurinribosid	10	12.6 ± 0.5 (9)	**
6-Mercaptopurinribosid	30	9.3 ± 0.5 (9)	**
BM 92.0729	30	15.4 ± 0.9 (9)	
BM 92.0729	100	13.0 ± 0.6 (9)	

[a] mean + SEM; Treatment once daily p.o., day 0-day+3
Sacrifice on day +4

* $p \leq 0.05$
** $p \leq 0.01$
} Mann-Whitney-test

Example 9

Bone marrow toxicity of BM 92.0729, Azathioprine, 6-Mercaptopurine, 6-Mercaptopurinribosid and Cyclosporin A in female Balb/c mice: Day + 4 (Exp. 940026).

Exp. 940026 is an experiment which was aimed at reproducing the results obtained in Exp. 930740 (Example 8). In this experiment Cyclosporin A was included as a reference compound, too. The outcome of the Exp. 940026 confirmed the results obtained in Exp. 930740 in vivo.

Bone marrow toxicity of BM 92.0729, Azathioprine, 6-Mercaptopurine, 6-Mercaptopurinribosid and Cyclosporin A in female Balb/c mice: Day + 4 (Exp. 940026)

Compound	Dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	Cells/femur (10^6)	
Control (0,5% Tylose)	-	15.6 ± 0.8 (10)	a
Azathioprine	10	11.1 ± 0.6 (10)	**
Azathioprine	30	9.1 ± 0.5 (10)	**
6-Mercaptopurine	10	10.9 ± 0.9 (10)	*
6-Mercaptopurine	30	6.2 ± 0.5 (10)	**
6-Mercaptopurinribosid	10	13.7 ± 1.4 (10)	*
6-Mercaptopurinribosid	30	8.4 ± 0.4 (10)	**
BM 92.0729	30	14.3 ± 0.5 (10)	
BM 92.0729	100	13.0 ± 0.4 (10)	
Cyclosporin A	5	13.1 ± 0.4 (10)	
Cyclosporin A	10	7.6 ± 1.4 (10)	**

[a] mean + SEM; Treatment once daily p.o., day 0-day+3
Sacrifice on day +4

* $p \leq 0.05$
** $p \leq 0.01$
} Mann-Whitney-test

Example 10

Bone marrow toxicity (µM) of BM 92.0700 and 5-FU in CFU-E and CFU-GM assays.

The table shown in Encl. 4 gives the mean IC_{50} values for 5-Fluorouridine (5-FU) and the 5-FU ether lipid conjugate BM 92.0700 for bone marrow toxicity in vitro in CFU-E and CFU-GM assays. For assay conditions, please refer to description of Encl. 1.

The data indicate that the ether lipid conjugate of 5-Fluorouridine BM 92.0700 is 610 times and 238 times less toxic on erythrocyte and granulocyte/macrophage bone marrow stem cells, respectively, compared to 5-FU itself.

Bone marrow toxicity (μM) of BM 92.0700 and 5-FU in CFU-E and CFU-GM assays

Compound	CFU-E[a]		CFU-GM[a]	
BM 92.0700	0.372 (3)	610 x	1.178 (7)	238 x
5-FU	0.00061 (3)		0.00496 (10)	

[a] **Mean; n, number of experiments**

Example 11

Influence of the 5-FU ether lipid conjugate BM 92.0700 (Fig. 1) and of 5-FU (Fig. 2) on the L 1210 leukemia in vivo: Survival time.

Mice were inoculated with L 1210 leukemia cells on day 0 (n = 10 animals/group) and were then treated once daily i.p. from day 0 (+1h) - day +41 (6 weeks) with the weekly cycles indicated on Encl. 5 and 6, respectively.

From the survival curves of the control and treatment groups shown on Encl. 6 it is obvious, that 5-FU has, as reported in the literature, a very narrow dose-efficacy profile, i.e. icreasing the dose, for example from 2 x 10/5 x 0.1 $mg \cdot kg^{-1} \cdot day^{-1}$ to 2 x 10/5 x 0.3 $mg \cdot kg^{-1} \cdot day^{-1}$ or to even higher doses lead to reduced survival rates.

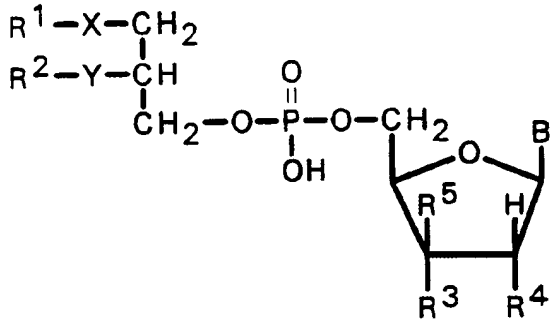
In contrast, with the 5-FU ether lipid conjugate BM 92.0700 a clear dose-dependent increase in survival time was obtained compared to control I and II (Fig. 1) indicating

that equimolar doses of BM 92.0700 are clearly more effective in this leukemia model compared to the standard compound 5-FU.

Taken into consideration that BM 92.0700 is more effective (Fig. 1 and 2) and much less toxic on bone marrow cells it can be concluded that BM 92.0700 has a much higher therapeutic index/ratio compared to the standard cytostatic 5-FU.

C L A I M S

1. Nucleoside monophosphate derivatives of formula (I)



(I),

wherein

R^1 may be a straight-chain or branched, saturated or unsaturated alkyl chain having 1-20 carbon atoms, optionally mono- or polysubstituted by halogen, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkylsulfinyl, or C_1-C_6 alkylsulfonyl groups;

R^2 may be hydrogen, a straight-chain or branched, saturated or unsaturated alkyl chain having 1-20 carbon atoms, optionally mono- or polysubstituted by halogen, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, C_1-C_6 alkoxycarbonyl, or C_1-C_6 alkylsulfonyl groups;

R^3 represents hydrogen, hydroxy, azido, amino, cyano, or halogen;

R^4 represents hydroxy, azido, amino, cyano, or halogen;

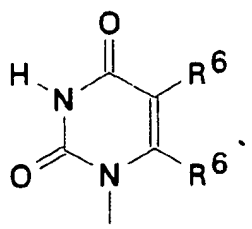
R^5 represents hydrogen, hydroxy, azido, amino, cyano, or halogen;

X represents a valence dash, oxygen, sulfur, a sulfinyl or sulfonyl group;

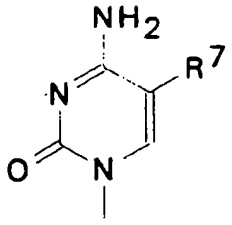
Y is a valence dash, an oxygen or sulfur atom;

B represents a purine and/or pyrimidine base of formula III(a-d)

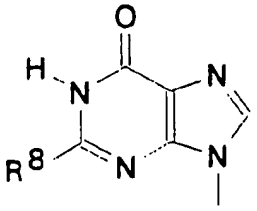
wherein



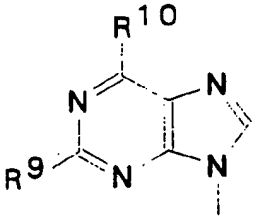
(IIIa)



(IIIb)



(IIIc)



(IIId)

R^6 may be hydrogen; an alkyl chain having 1-4 carbon atoms, which may be substituted by halogen; an alkenyl and/or alkinyl residue having 2-6 carbon atoms, optionally substituted by halogen; or halogen;

$R^{6'}$ may be a hydrogen atom or a benzyl or phenylthio residue;

R^7 may be hydrogen; an alkyl chain having 1-4 carbon atoms, which may be substituted by halogen; or halogen;

R^8 may be hydrogen, an alkyl chain having 1-4 carbon atoms, halogen, or a hydroxy or an amino group;

R^9 may be hydrogen, an amino group or a halogen atom; and

R^{10} may be hydrogen, halogen, mercapto, hydroxy, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, or an amino group which may be mono- or disubstituted by C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy-C_2-C_6 alkyl, and/or C_3-C_6 cycloalkyl, aryl, hetaryl, aralkyl, or hetarylalkyl groups, optionally substituted at the aryl or hetaryl residue by one or more mercapto, hydroxy, C_1-C_6 alkoxy, or C_1-C_6 alkyl groups or halogen; or C_2-C_6 alkenyl optionally substituted by mono- or dialkyl or alkoxy groups;

with the proviso that at least one of the residues R^3 or R^5 is hydrogen;

to their tautomers, their optically active forms and racemic mixtures, and to their physiologically acceptable salts of inorganic and organic acids or bases.

2. Nucleoside monophosphate derivatives of formula I according to claim 1, characterized in that R^1 represents a straight-chain C_8-C_{15} alkyl group which may be substituted by a C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto group.

3. Nucleoside monophosphate derivatives of formula I according to claim 1 or 2, characterized in that

R^2 represents a straight-chain C_8-C_{15} alkyl group which may further be substituted by a C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto group.

4. Nucleoside monophosphate derivatives of formula I according to one of claims 1-3, characterized in that X represents sulfur, sulfinyl or sulfonyl, and Y represents oxygen.

5. Nucleoside monophosphate derivatives of formula I according to one of claims 1-3, characterized in that X and Y represent a valence dash, R^2 is hydrogen, and R^1 represents a C_1-C_{20} alkyl chain which optionally may be substituted by C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto.

6. Nucleoside monophosphate derivatives of formula I according to one of claims 1-5, characterized in that R^5 represents hydrogen, azido, cyano, or halogen.

7. Nucleoside monophosphate derivatives of formula I according to one of claims 1-6, characterized in that R^3 or R^4 represent a hydroxy, cyano or azido group or a halogen atom, wherein the residues may be the same or different.

8. Nucleoside monophosphate derivatives of formula I according to one of claims 1-7, characterized in that R^6 and R^7 represent a hydrogen atom; a C_1-C_6 alkyl residue optionally substituted by halogen; or a halogen atom; or a C_2-C_6 alkenyl or alkinyl group optionally substituted by halogen, wherein the residues may be the same or different.

9. Nucleoside monophosphate derivatives of formula I according to one of claims 1-8, characterized in

that R^8 represents a hydrogen atom, a C_1-C_6 alkyl residue, an amino group, or a halogen atom.

10. Nucleoside monophosphate derivatives of formula I according to one of claims 1-9, characterized in that R^{10} represents a hydrogen or halogen atom, a C_1-C_6 alkoxy, mercapto, C_1-C_6 alkylmercapto, or an amino group which may be mono- or disubstituted by a C_1-C_6 alkyl or a hydroxy-C_2-C_6 alkyl, or by a C_3-C_6 cycloalkyl, aryl or aralkyl residue, which optionally may further be substituted by one or more hydroxy, C_1-C_6 alkoxy or C_1-C_6 alkyl groups or by halogen atoms.

11. Nucleoside monophosphate derivatives of formula I according to one of claims 1-10, characterized in that

R^1 represents a straight-chain C_9-C_{13} alkyl group optionally further substituted by a methoxy, ethoxy, butoxy, hexyloxy, methylmercapto, ethylmercapto, propylmercapto, butylmercapto, or hexylmercapto residue;

R^2 represents a straight-chain C_8-C_{14} alkyl group optionally further substituted by a methoxy, ethoxy, propoxy, butoxy, hexyloxy, methylmercapto, ethylmercapto, butylmercapto, or hexylmercapto residue;

R^3 represents a hydroxy, cyano, azido or fluorine residue;

R^4 represents a hydroxy, cyano, azido or fluorine residue;

R^5 represents hydrogen;

R^6 represents hydrogen, a methyl, trifluoromethyl, ethyl, vinyl, propenyl, ethinyl, propinyl residue, or a fluorine, chlorine or bromine atom;

$R^{6'}$ represents hydrogen;

R^7 represents hydrogen, a methyl, ethyl, vinyl, propenyl, ethinyl, propinyl residue, or a chlorine or bromine atom;

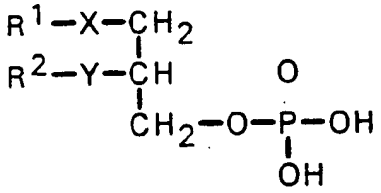
and/or

R^{10} represents hydrogen, a methoxy, ethoxy, propoxy, butoxy, hexyloxy, mercapto, methylmercapto, ethylmercapto, butylmercapto, hexylmercapto residue, an amino group optionally substituted by thienyl, furyl, pyridyl, thienylmethyl, furylmethyl, or pyridylmethyl.

12. Nucleoside monophosphate derivatives of formula I according to one of claims 1-11 characterized in that the nucleoside portion is selected from the following group: 6-mercaptopurine-9-ß-D-ribofuranoside, 5-fluorouridine, inosine, 5-methyluridine, 2',3'-didesoxy-2',3'-difluorothymidine, 5-chlorouridine, 5-trifluoromethyluridine, 5-ethinyluridine, 5-ethinylcytidine, 5-prop-1-enyluridine, 5-prop-2-enyluridine, adenosine, guanosine, 2,6-diaminopurine-9-ß-D-ribofuranoside, 2-amino-6-mercaptopurine-9-ß-D-ribofuranoside, 2-amino-6-methylmercaptopurine-9-ß-D-ribofuranoside, 2-amino-6-chloropurine-9-ß-D-ribofuranoside, 2'-desoxy-2'-aminoadenosine, 2'-desoxy-2'-azidoadenosine, 2'-desoxy-2'-azidocytidine, 2'-desoxy-5-fluorouridine, 2-chloroadenosine, 2-fluoroadenosine, 3'-desoxy-5-fluoroadenosine, 6-methylmercaptopurine-9-ß-D-ribofuranoside, 2-bromoadenosine, 2-fluoro-2'-desoxyadenosine.

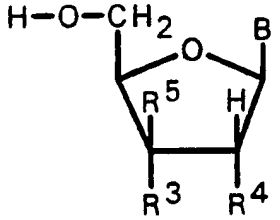
13. A process for the preparation of nucleoside monophosphate derivatives of formula I according to one of claims 1-12, characterized by

1. reacting a compound of general formula V



(V),

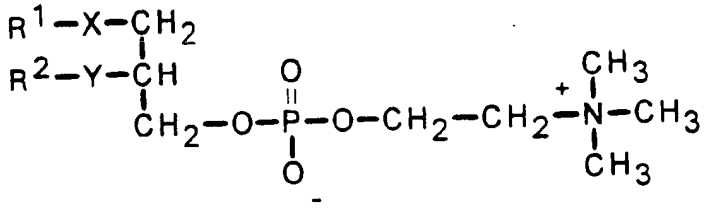
wherein R^1, R^2, X and Y have the meanings as indicated, with a compound of general formula VI



(VI),

wherein R^3, R^4, R^5 and B have the above mentioned meanings, or represent a hydroxy group protected by an oxygen protecting group familiar to the artisan, in the presence of an activating acid chloride, such as 2,4,6-triisopropylbenzenesulfonic acid chloride, and a tertiary nitrogen base, e.g., pyridine or lutidine, in an inert solvent, such as toluene, or immediately in anhydrous pyridine, and optionally, subsequent to hydrolysis, removing the oxygen protecting groups according to procedures conventional in nucleoside chemistry, or

2. reacting a compound of general formula VII



(VII),

wherein R^1, R^2, X and Y have the above-mentioned meanings, with a compound of general formula VI, wherein R^3, R^4, R^5 and B have the above-mentioned meanings, in the presence of phospholipase D from *Streptomyces* in an inert solvent such as chloroform, in the presence of a suitable buffer, and optionally, subsequent to reaction, removing the oxygen protecting groups according to procedures conventional in nucleoside chemistry.

14. A drug, containing at least one compound of formula I according to one of claims 1-12, as well as pharmaceutical adjuvants or vehicles.

15. Use of compounds of formula I according to one of claims 1-12 for the preparation of drugs for the treatment of tumors or human diseases.

Fig. 1

Influence of BM 92.0700 on the L1210 leukemia in vivo:

Survival time (Exp. 950001)

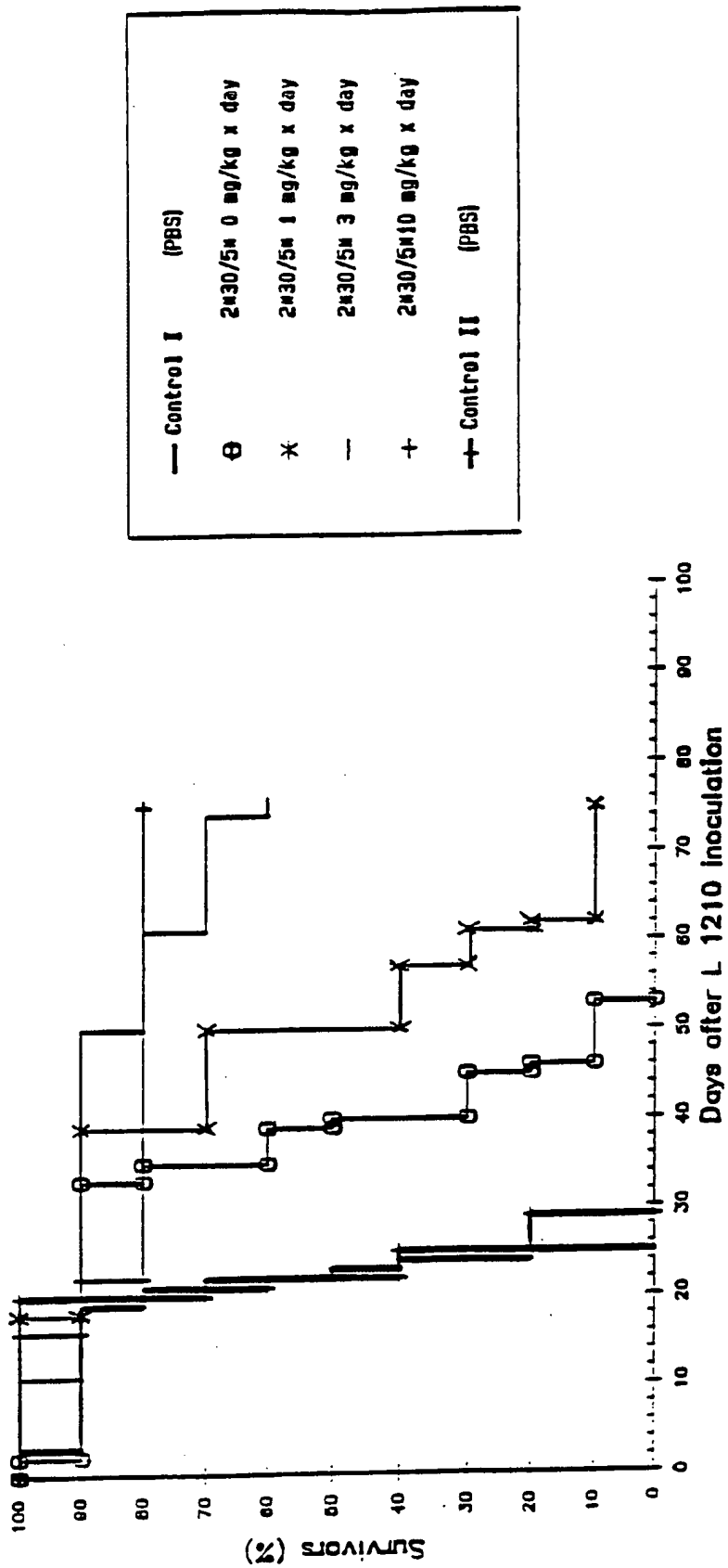
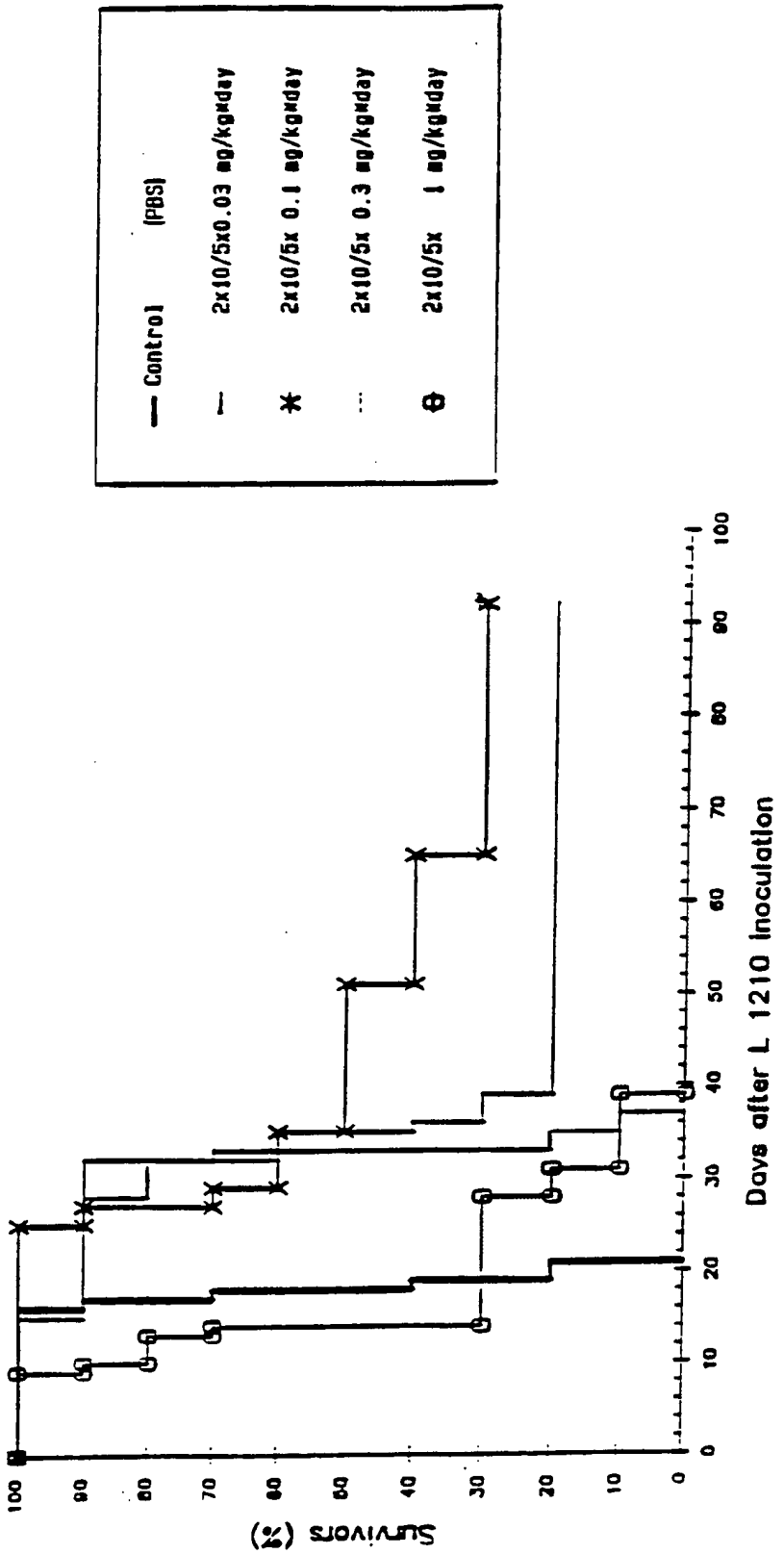


Fig. 2

Influence of 5-FU on the L1210 leukemia in vivo:

Survival time (Exp. 950066, Part II)



INTERNATIONAL SEARCH REPORT

Interna: I Application No
PCT/EP 95/01951

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H19/10 C07H19/207 C07H19/04 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP-A-0 122 151 (MEITO SANGYO KK) 17 October 1984 see pages 8, 44, 53 and claim 1 ---	1-3,6-13
X,Y	EP-A-0 262 876 (TOYO JOZO KK) 6 April 1988 see the whole document ---	1-3,6-15
X	PATENT ABSTRACTS OF JAPAN vol. 012 no. 326 (C-525) ,5 September 1988 & JP,A,63 091090 (TOYO JOZO CO LTD) 21 April 1988, see abstract ---	1-3,6-13
Y	WO-A-93 16091 (BOEHRINGER MANNHEIM GMBH) 19 August 1993 see claim 1 ---	4,5
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 September 1995	10. 10. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Bardili, W

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	EP-A-0 306 845 (HOECHST AG) 15 March 1989 see page 4, line 48 - line 53; claims 1-4	1-3, 6-12,14, 15
Y	DE-A-29 30 904 (GAURI KAILASH KUMAR DR) 19 February 1981 see claim 1	1-3, 6-12,14, 15
Y	WO-A-92 18520 (KNOLL AG) 29 October 1992 see the whole document	1-3,6-15
A	US-A-4 797 479 (SHUTO SATOSHI ET AL) 10 January 1989 see the whole document	1-15
Y	WO-A-92 03462 (BOEHRINGER MANNHEIM GMBH) 5 March 1992 cited in the application see claim 1	4,5

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